



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



American Society of Hematology
2021 L Street NW, Suite 900,
Washington, DC 20036
Phone: 202-776-0544 | Fax 202-776-0545
editorial@hematology.org

Neutralization of SARS-CoV-2 Omicron after vaccination of myelodysplastic syndromes and acute myeloid leukemia patients

Tracking no: BLD-2022-016087R1

Lorenza Bellusci (Food and Drug Administration, United States) Gabrielle Grubbs (Food and Drug Administration, United States) Pragya Srivastava (Roswell Park Comprehensive Cancer Center, United States) Michael Nemeth (Roswell Park Comprehensive Cancer Institute, United States) Elizabeth Griffiths (Roswell Park Comprehensive Cancer Center, United States) Hana Golding (Center for Biologics Evaluation and Research, U. S. Food and Drug Administration, United States) Surender Khurana (Food and Drug Administration, United States)

Abstract:

Conflict of interest: No COI declared

COI notes:

Preprint server: No;

Author contributions and disclosures: Author Contributions: Designed research: S.K., H.G. and E.G. Clinical specimens and unblinded clinical data: M.N and E.G. Performed assays: L.B., G.G. and, S.K. Contributed to Writing: S.K., H.G., M.N., and E.G.

Non-author contributions and disclosures: No;

Agreement to Share Publication-Related Data and Data Sharing Statement: Data sharing. All data needed to evaluate the conclusions in the article are present in the manuscript.

Clinical trial registration information (if any):

Neutralization of SARS-CoV-2 Omicron after vaccination of myelodysplastic syndromes and acute myeloid leukemia patients

Lorenza Bellusci, PhD¹; Gabrielle Grubbs, BS¹; Pragya Srivastava, PhD²; Michael J. Nemeth, PhD³; Elizabeth A. Griffiths, MD^{2,4}; Hana Golding, PhD¹ and Surender Khurana, PhD^{1*}

¹ Division of Viral Products, Center for Biologics Evaluation and Research (CBER), FDA, Silver Spring, Maryland, 20993, USA.

²Departments of Medicine, ³Immunology, ⁴Pharmacology and Therapeutics, Roswell Park Comprehensive Cancer Center, Buffalo, New York, 14263, USA

***Corresponding author:**

*Surender Khurana, Ph.D.

Division of Viral Products,

Center for Biologics Evaluation and Research (CBER)

Food and Drug Administration (FDA)

10903 New Hampshire Avenue

Silver Spring, MD, USA 20993

Phone- 240-402-9632, Fax- (301) 595-1125

E. mail- Surender.Khurana@fda.hhs.gov

22

23 **To the Editor:**

24 Despite vaccination against SARS-CoV-2, there is increased risk of breakthrough
25 infections among persons with immune dysfunction such as those with autoimmune
26 disease on immunosuppression, and individuals with chronic inflammation, B cell
27 lymphoma, or solid organ transplants¹. Breakthrough infections are of particular concern
28 among patients with hematologic malignancy who have been demonstrated to fare
29 particularly poorly when infected with SARS-CoV-2².

30 Myeloid neoplasms such as myelodysplastic syndromes (MDS), acute myeloid
31 leukemia (AML), and myeloproliferative neoplasms are often observed in older persons,
32 and can share similar disease ontogeny. Infectious complications are a key contributor
33 to mortality for patients with these conditions³⁻⁷. Patients with myeloid neoplasms are
34 underrepresented in studies of response to SARS-CoV-2 vaccines^{3,8-10}. Although
35 prioritized for early vaccination, to our knowledge the ability of patients with myeloid
36 neoplasms such as MDS to elicit neutralizing antibodies against the ancestral strain and
37 variants of concern (VOCs) has not been reported to date.

38 We evaluated a cohort of patients with myeloid neoplasms (n=48; median age,
39 70 years; range, 28-89 years) receiving standard therapies [e.g., supportive care,
40 growth factors, DNA hypomethylating agents (HMA), or kinase inhibitors] for their
41 neutralizing antibody responses to vaccine-homologous SARS-CoV-2 WA1/2020 strain
42 and five VOCs in the periods following second and third (booster) vaccinations (Table
43 1). Two patients had documented breakthrough SARS-CoV-2 infection after two vaccine
44 doses, with one patient (P-8) had prolonged hospitalization but ultimately recovered,

and a second patient (P-30) with mild symptoms and was managed as an outpatient (Supplementary Table S1). After the third vaccine dose, one patient (P-53) had documented breakthrough SARS-CoV-2 infection resulting in hospitalization who recovered fully. Supplementary Table S1 lists the extended clinical characteristics, treatment summary, vaccine/booster type, and the time point of sampling relative to COVID-19 vaccination. Healthy health-care workers (n=16) working at research institution and who were neither exposed to SARS-CoV-2 and do not work with COVID-19 patients were used as the comparative control cohort (median age, 34.5 years; range 21-75 years). None of the healthy controls had breakthrough SARS-CoV-2 infections.

We performed SARS-CoV-2 virus neutralization assays, which in contrast to conventional assays that measure SARS-CoV-2 binding antibodies, can distinguish the capacity of immune sera to block cell entry by the prototype WA1/2020 strain, used in the vaccines, as well as individual VOCs. Virus neutralization titers have been correlated with protection against SARS-CoV-2 infections and especially against severe disease. Post-vaccination sera were evaluated in a qualified SARS-CoV-2 pseudovirion neutralization assay (PsVNA) using SARS-CoV-2 WA1/2020 strain and the five VOC strains: Alpha, Beta, Gamma, Delta, and Omicron (see supplemental methods). SARS-CoV-2 neutralizing activity measured by PsVNA as 50% neutralization titers (PsVNA₅₀) correlated with PRNT (plaque reduction neutralization test) with authentic SARS-CoV-2 virus in our previous studies¹¹. The median time intervals between vaccination and evaluation of sera in MDS/AML patients were 150.5 days following the 2nd vaccination (n=38) and 30.5 days following the 3rd vaccination (n=11), while for healthy controls, it

was 38 days after 2nd vaccination (n=16) and 57.5 days after the 3rd vaccination (n=16) (Table 1).

After two vaccinations, the control group demonstrated a robust response (100% with PsVNA50 >1:40) against the vaccine homologous WA1/2020 [PsVNA50 geometric mean titers (GMT) of 1:1713]. In contrast, patients with myeloid neoplasms (n=38) displayed significantly weaker neutralization titers with GMT of 1:139 (7/38 non-responders with PsVNA50 of <1:20) (Fig. 1A and Supplementary Table S2). We did not observe significant differences in vaccine response between patients with AML (n = 9), MDS (n = 17), or MPN (n = 12) after two vaccine doses.

Booster (third) vaccination in the healthy controls resulted in consistently strong neutralizing antibody responses against the WA1/2020 strain (PsVNA50 of >1:500, GMT of 1:3141). In contrast, among 11 patients with myeloid neoplasms (diagnosed as MDS or AML) who received three vaccine doses, WA1/2020 neutralizing antibodies were highly variable (GMT of 1:304), with 2/11 demonstrating no neutralization response (PsVNA50 of <1:20), and only 4/11 strong responders (PsVNA50 of >1:500) against WA1/2020 (Fig. 1B and Supplementary Table 2).

In healthy adults, two vaccinations demonstrated 1.3, 3.5, 3.4, 1.8-fold reduction against Alpha, Beta, Gamma, and Delta variants, respectively, and more pronounced loss of activity (38.9-fold) against Omicron, compared with the vaccine-homologous WA1/2020 (Fig. 1A and Supplementary Table S2). Following a third vaccine dose, neutralization titers in the healthy cohort increased modestly against Alpha (1.7-fold), Beta (2.1-fold), Gamma (2.3-fold), and Delta (2.0-fold) variants compared with the second vaccination. Moreover, third vaccination improved neutralization titer against

Omicron (GMT of 1:334) by 7.6-fold compared with antibody response following the second vaccination (GMT of 1:44); but still the neutralizing antibodies GMT against Omicron was reduced by 9.4-fold relative to WA1/2020 (Fig. 1B and Supplementary Table S2).

In contrast to healthy controls, the majority of patients with myeloid neoplasms demonstrated minimal or no neutralizing antibodies against the VOCs including Omicron (92% patients with PsVNA50 <1:20 against Omicron) after two vaccinations (Fig. 1A and Supplementary Table S2), except for patient P-19 (82-year-old female with MDS). Among the patients who received three vaccinations, the majority (7/11) exhibited much lower neutralization responses against all VOC and no neutralization titers against Omicron compared with healthy controls (Fig. 1B). Even the four patients that exhibited strong anti-WA1/2020 responses (P-52, P-54, P-55, and P-30R; PsVNA50 > 1:1000) demonstrated profoundly lower responses against Omicron (PsVNA50 of 1:52, 1:30, 1:169 and 1:257, respectively) after the third vaccination (Supplementary Table S2). The patient (P-30R) with highest neutralization titer (1:257) against Omicron after third vaccination had breakthrough SARS-CoV-2 infection after second vaccination.

The low neutralization titers observed in MDS/AML against both WA1/2020 and VOCs did not provide a complete picture regarding their antibody response to vaccination. Therefore, we also measured IgG binding to the SARS-CoV-2 spike Receptor binding domain (RBD) derived from vaccine-homologous WA1/2020 as well as Omicron variant using ELISA (Fig. 1C-D). Binding to both WA1 and Omicron RBD was robust for the healthy adults after two and three vaccinations (Fig. 1C-D). In

comparison, after 2nd vaccination the RBD-binding antibodies from MDS/AML patients were more variable, with 11 of 38 patients demonstrating RBD-IgG end-point titers below 1:200 serum dilution against the Omicron RBD and lower than the healthy cohort (Fig. 1C). MDS/AML patients receiving three vaccinations showed increased antibody binding titers against the Omicron RBD, but they were still lower than the healthy adults (Fig. 1D). A correlation was observed between SARS-CoV-2 RBD-binding antibody titers and SARS-CoV-2 neutralization titers for these two cohorts that received either two or three vaccinations (Supplementary Fig. S1).

Large scale vaccine effectiveness studies evaluating clinical outcomes and complications of COVID-19 infections demonstrated slightly lower effectiveness in persons with coexisting conditions¹². The correlates of protection for SARS-CoV-2 strains prior to circulation of antibody-resistant Omicron variant, suggested neutralizing antibody titers above 1:60 can reasonably provide protection against severe COVID-19^{13,14}. Based on these studies, the healthy individuals who received three doses of the mRNA vaccines are likely to be protected against severe disease from the Omicron variant. It is possible other components of immune system in addition to neutralizing antibodies including T cells can contribute to protection from severe disease. However, the effectiveness of two doses of mRNA vaccine in patients with hematological neoplasms was found to be significantly reduced after mass vaccination in Israel, showing 1.7-2.3 increased risk of symptomatic disease, hospitalization, and death¹⁵.

Patients with myeloid neoplasms, especially high-risk diseases such as MDS and AML who are often treated with similar HMA-based chemotherapy regimens, are underrepresented in the published literature reporting the efficacy of SARS-CoV-2

vaccination. Our findings demonstrate that mRNA vaccination in a cohort of patients with myeloid neoplasms, both untreated and receiving standard of care therapeutics, elicited variable titers of RBD-binding antibodies. But most of these patients had low (or none) neutralizing antibody responses against the Omicron variant following two or three COVID-19 vaccinations that should be confirmed in larger studies. A recent study by Mori *et al.* reported similar seroconversion rates in healthy controls and patients with MDS/AML, especially those not on treatment, after mRNA vaccination¹⁶. However, only anti-spike binding antibodies were measured in the study. Our study underscores the importance of measuring virus neutralization titers, both against the vaccine strain and against clinically relevant circulating VOCs such as Omicron.

These observations highlight the immunodeficiency in this patient population. Even patients with MDS on observation generated weak neutralizing antibody response following SARS-CoV-2 vaccination. These patients are likely to be at increased risk for breakthrough infection, especially from Omicron, and therefore should be prioritized for post-exposure treatments early after SARS-CoV-2 infection.

Acknowledgements: We thank Basil Golding and Keith Peden at FDA, and Philip McCarthy at Roswell Park Comprehensive Cancer Center for review of the manuscript. We thank Carol Weiss (FDA) and NIH Vaccine Research Center for providing plasmid clones expressing SARS-CoV-2 spike variants. Study was funded by the NIAID IAA number AA121031 and National Cancer Institute (NCI) grant number P30CA016056.

Funding: Antibody response study was supported by FDA's MCMi grant #OCET 2021-1565, to S.K and NIAID IAA AA121031, and donations from the family of Kathleen Wieczkowski and Robert Drajem and the Fighting Irish Fighting Cancer Team *via* the Roswell Park Alliance Foundation to EAG. This work was supported by National Cancer Institute (NCI) grant P30CA016056 involving the use of Roswell Park Comprehensive Cancer Center's Hematologic Procurement Shared Resource. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Author Contributions:

Designed research: S.K., H.G. and E.G.

Clinical specimens and unblinded clinical data: M.N and E.G.

Performed assays: L.B., G.G. and, S.K.

Contributed to Writing: S.K., H.G., M.N., and E.G.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

179 **Data sharing.** All data needed to evaluate the conclusions in the article are present in
180 the manuscript.

181

References

1. Ghione P, Gu JJ, Attwood K, et al. Impaired humoral responses to COVID-19 vaccination in patients with lymphoma receiving B-cell-directed therapies. *Blood*. 2021;138(9):811-814.
2. Vijenthira A, Gong IY, Fox TA, et al. Outcomes of patients with hematologic malignancies and COVID-19: a systematic review and meta-analysis of 3377 patients. *Blood*. 2020;136(25):2881-2892.
3. Dayyani F, Conley AP, Strom SS, et al. Cause of death in patients with lower-risk myelodysplastic syndrome. *Cancer*. 2010;116(9):2174-2179.
4. Sakatoku K, Takeoka Y, Miura A, et al. Combination of Frailty Status and Comorbidity Score Improves the Stratification of Survival in Patients With Myelodysplastic Syndrome Owing to Good Predictive Capability for Infection-related Mortality. *Clin Lymphoma Myeloma Leuk*. 2019;19(12):799-805.
5. Trubiano JA, Dickinson M, Thursky KA, et al. Incidence, etiology and timing of infections following azacitidine therapy for myelodysplastic syndromes. *Leuk Lymphoma*. 2017;58(10):2379-2386.
6. Pagano L, Caira M. Risks for infection in patients with myelodysplasia and acute leukemia. *Curr Opin Infect Dis*. 2012;25(6):612-618.
7. Landt-blom AR, Andersson TM, Dickman PW, et al. Risk of infections in patients with myeloproliferative neoplasms-a population-based cohort study of 8363 patients. *Leukemia*. 2021;35(2):476-484.
8. Griffiths EA, Segal BH. Immune responses to COVID-19 vaccines in patients with cancer: Promising results and a note of caution. *Cancer Cell*. 2021;39(8):1045-1047.
9. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127(20):2391-2405.
10. Li Z, Philip M, Ferrell PB. Alterations of T-cell-mediated immunity in acute myeloid leukemia. *Oncogene*. 2020;39(18):3611-3619.
11. Tang J, Lee Y, Ravichandran S, et al. Epitope diversity of SARS-CoV-2 hyperimmune intravenous human immunoglobulins and neutralization of variants of concern. *iScience*. 2021:103006.
12. Dagan N, Barda N, Kepten E, et al. BNT162b2 mRNA Covid-19 Vaccine in a Nationwide Mass Vaccination Setting. *N Engl J Med*. 2021;384(15):1412-1423.
13. Gilbert PB, Montefiori DC, McDermott AB, et al. Immune correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy clinical trial. *Science*. 2021:eab3435.
14. Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med*. 2021;27(7):1205-1211.
15. Mittelman M, Magen O, Barda N, et al. Effectiveness of the BNT162b2mRNA Covid-19 Vaccine in Patients with Hematological Neoplasms. *Blood*. 2021.
16. Mori A, Onozawa M, Tsukamoto S, et al. Humoral response to mRNA-based COVID-19 vaccine in patients with myeloid malignancies. *Br J Haematol*. 2022.

Table 1. Summary of clinical characteristics of healthy controls and patients with myeloid malignancies

Characteristic	Myeloid Patients (N=48)	Healthy Controls (N=16)
Age (years)	48 (28-89)	34.5 (21-75)
Sex	27 Male/ 21 Female	5 Male / 11 Female
Diagnosis		
MDS	N=23	
AML	N=13	
CMML	N=1	
Jak2 ^{V617F+} MPN		
ET	N=1	
PV	N=6	
MF	N=4	
Treatment		
Observation, GF	N=16	
HMA (Azacitidine or Decitabine) Monotherapy	N=7	
HMA/Venetoclax	N=10	
HMA/Other	N=4	
Allo-BMT	N=2	
Peginterferon	N=1	
Hydroxyurea	N=3	
Ruxolitinib	N=3	
Ruxolitinib/Hydroxyurea	N=1	
Anagrelide	N=1	
Vaccines/Boosters		
Moderna (2nd Vaccine)	N=19	N=8
Pfizer (2nd Vaccine)	N=18	N=8
Janssen (1 Vaccine)	N=1	
Same mRNA 2nd & 3rd vaccine	N=9	N=8
Mixed mRNA 2nd & 3rd vaccine (Moderna-Pfizer)	N=1	N=8
Documented breakthrough SARS-CoV-2 infection	N=3	
Median time of sample collection from 2nd vaccine dose	150.5 days	38 days
Median time of sample collection from 3rd vaccine dose	30.5 days	57.5 days

220

221

Figure Legend

Figure 1: Neutralization and antibody binding of post-vaccination serum against SARS-CoV-2 WA1/2020 strain and variants of concern.

Neutralization assays were performed with the use of pseudoviruses expressing the SARS-CoV-2 spike proteins of the WA1/2020 vaccine strain or the Alpha, Beta, Gamma, Delta or the Omicron variants. (Panel A) Serum samples following two doses of SARS-CoV-2 mRNA vaccination were obtained from 38 AML/MDS patients (P; in red) or 16 healthy controls (C; in blue). (Panel B) Post-third vaccination samples were obtained from 11 AML/MDS patients and 16 healthy controls. The heights of the bars and the numbers over the bars indicate the geometric mean titers (GMT), and the whiskers indicate 95% confidence intervals, and are color-coded. The assay of each serum sample was performed in duplicate. Each data point represents an individual sample (circles) and indicates the 50% neutralization titer obtained with each sample against the corresponding pseudovirus. The horizontal dashed line indicates the limit of detection for the neutralization assay (PsVNA50 of 20). The raw data and information regarding the serum samples from vaccinated participant (sex, age, vaccine type and samples collected post-vaccination and 50% neutralization titers against various SARS-CoV-2 strains) are summarized in Supplementary Table S2 in the Supplementary Appendix. Differences between SARS-CoV-2 strains were analyzed by lme4 and emmeans packages in R using Tukey's pairwise multiple comparison test and the p-values are shown. (Panels C-D) SARS-CoV-2 receptor binding domain (RBD) binding IgG to vaccine-homologous WA1/2020 and Omicron variant in serum samples following two doses of SARS-CoV-2 mRNA vaccination (panel C) from 38 AML/MDS patients (P;

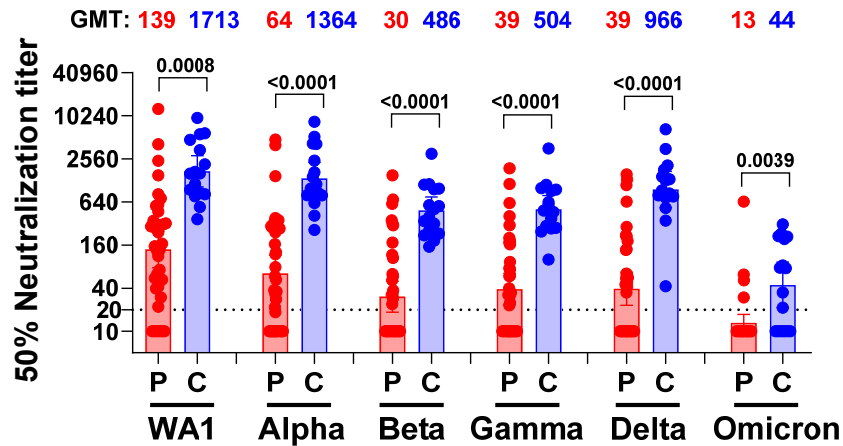
245 in red) and 16 healthy controls (C; in blue) or following three doses of vaccination (panel
246 D) from 11 AML/MDS patients (P; in red) and 16 healthy controls (C; in blue). Each
247 serum sample was evaluated in IgG-ELISA in duplicate to determine the RBD-binding
248 IgG end-point titer against RBD of either WA1/2020 or the Omicron variant. The height
249 of bars and numbers over the bars indicate the IgG GMTs, and the whiskers indicate
250 95% confidence intervals. The horizontal dashed line indicates the limit of detection for
251 IgG ELISA (1:100). Statistical differences between patients and controls were analyzed
252 by lme4 and emmeans packages in R using Tukey's pairwise multiple comparison test
253 and the p-values are shown.

Table 1. Summary of clinical characteristics of healthy controls and patients with myeloid malignancies

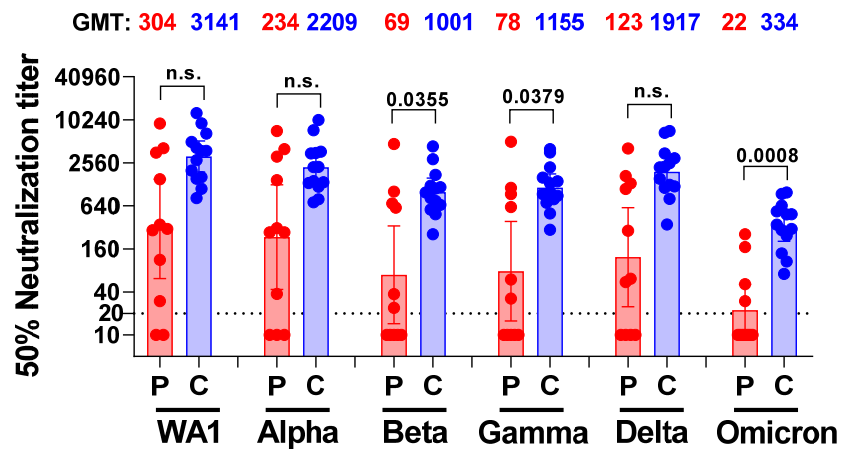
Characteristic	Myeloid Patients (N=48)	Healthy Controls (N=16)
Age (years)	48 (28-89)	34.5 (21-75)
Sex	27 Male/ 21 Female	5 Male / 11 Female
Diagnosis		
MDS	N=23	
AML	N=13	
CMMML	N=1	
Jak2 ^{V617F+} MPN		
ET	N=1	
PV	N=6	
MF	N=4	
Treatment		
Observation, GF	N=16	
HMA (Azacitidine or Decitabine) Monotherapy	N=7	
HMA/Venetoclax	N=10	
HMA/Other	N=4	
Allo-BMT	N=2	
Peginterferon	N=1	
Hydroxyurea	N=3	
Ruxolitinib	N=3	
Ruxolitinib/Hydroxyurea	N=1	
Anagrelide	N=1	
Vaccines/Boosters		
Moderna (2nd Vaccine)	N=19	N=8
Pfizer (2nd Vaccine)	N=18	N=8
Janssen (1 Vaccine)	N=1	
Same mRNA 2nd & 3rd vaccine	N=9	N=8
Mixed mRNA 2nd & 3rd vaccine (Moderna-Pfizer)	N=1	N=8
Documented breakthrough SARS-CoV-2 infection	N=3	
Median time of sample collection from 2nd vaccine dose	150.5 days	38 days
Median time of sample collection from 3rd vaccine dose	30.5 days	57.5 days

Figure 1

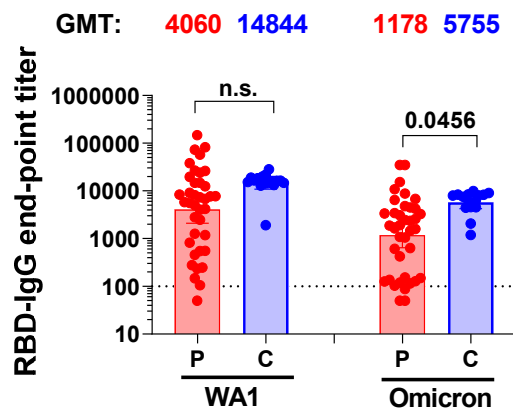
A. Neutralization Assay with 2nd-vaccine dose serum



B. Neutralization Assay with 3rd-dose vaccine serum



C. RBD ELISA: 2nd-vaccine



D. RBD ELISA: 3rd-vaccine

